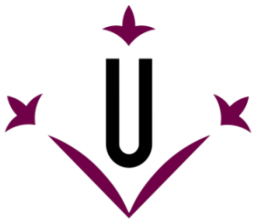



# Chitosan/tripolyphosphate nanoaggregates enhance the antibrowning effect of ascorbic acid on mushroom slices

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**Abstract**

Enzymatic browning is the main deleterious phenomenon affecting the quality of minimally processed mushrooms. Nano-encapsulation of antibrowning agents provides a new strategy to prevent discoloration in minimally processed commodities. In this study, coatings containing AA-loaded chitosan/tripolyphosphate nanoaggregates were applied to evaluate the postharvest preservation of minimally processed mushrooms during 16 d of storage at 5 °C. Package headspace composition, browning index, firmness, phenolic and ascorbic content, antioxidant capacity and polyphenol oxidase activity were evaluated as quality indicators. The results indicate that nanostructured coatings can effectively alleviate browning development (browning index was significantly reduced) and maintain firmness. Higher levels of phenolic compounds, ascorbic acid, as well as improvement in antioxidant capacity were achieved with the application of nano-encapsulated ascorbic acid. Polyphenol oxidase activity was partially inhibited as a consequence of the high AA concentration maintained during storage. The results allow concluding that AA-loaded chitosan/tripolyphosphate nanoaggregates are an interesting alternative to prevent browning of fresh-cut mushrooms and maintain high ascorbic acid concentrations through refrigerated storage.

**Keywords:** fresh-cut produce; mushrooms; nanoaggregates; browning; edible coatings

## 1. Introduction.

Fresh mushrooms (*Agaricus bisporus*) are highly perishable, with a shelf life of less than three days at ambient temperature and from eight to ten days under refrigeration conditions (Taghizadeh et al., 2010). Increased respiration rates, susceptibility to enzymatic browning and microbial spoilage are the main causes for shelf-life reduction (Mohapatra et al., 2010). Thermal processing is the most commonly used technology to preserve mushrooms. However, thermally treated commodities have lower nutritional value and sensory quality than fresh products due to the loss of antioxidants and other nutrients, together with changes in color, texture and flavor (Murcia et al., 2000).

Fresh-cut processing offers consumers highly nutritious, convenient and healthful commodities while maintaining the freshness of the non-processed products. However, the quality of minimally processed mushrooms is deleteriously affected by enzymatic browning on both external and cut surfaces (Oms Oliu et al., 2010). Enzymatic browning is catalyzed by polyphenol oxidase (PPO) and involves the oxidation of phenolic substrates into quinones. These compounds in turn undergo further reactions to yield dark-colored melanins that substantially curb the acceptance of the product by consumers. The major PPO enzyme responsible for browning in mushrooms is a tyrosinase (Jolivet et al., 1998). Several methods have been proposed to control browning phenomena in order to extend the shelf-life of fresh cut mushrooms, e.g. modified atmosphere packaging (Cliffe-Byrnes and O'Beirne, 2008), chemical dips (Sapers et al., 2001; Singh et al., 2010), ozone (Yuk et al., 2007), and irradiation (Beaulieu et al., 2002; Oms Oliu et al., 2010).

Ascorbic acid (AA) is one of the most widely used antibrowning agents for the prevention of browning in minimally processed commodities. Because of its reducing nature, it has been successfully applied in commercial mushrooms commodities (Sapers 1994, 2001),

avocados (Soliva-Fortuny et al.,2002), and apples (Piagentini et al., 2012). Nevertheless, significant amounts of the initial content are often lost during processing and storage because of the exposure to undesired temperatures, oxygen, light and other oxidizing agents (Alishahi et al., 2011). The enhancement of ascorbic acid stability has been reported to significantly extend browning inhibition in minimally processed products during storage, while improving its nutritional value (Gorny et al., 2002). Therefore the development of technologies that prevent ascorbic acid degradation in food processing is an interesting research field. Recent advances in nanoscience and nanotechnology offer a revolutionary approach to the development of novel and healthier foods. Nano-encapsulation has been utilized for shelf life extension of ascorbic acid and other compounds (Desai and Park, 2005). Moreover, it may be used to reduce the exposure of ascorbic acid to environmental factors such as temperature and oxygen (Jang and Lee, 2008).

Nanoparticle properties are highly dependent on the selected materials and the manufacturing conditions (Santiago and Castro, 2016). Chitosan is a hydrophilic, biocompatible and biodegradable polysaccharide. Due to its low toxicity, it is frequently used as a food additive. Chitosan nanoparticles can be easily produced by ionic gelation between cationic amino groups of chitosan and multivalent anions, such as tripolyphosphate (TPP) (Koo et al., 2011). Nano-encapsulation of ascorbic acid into chitosan nanoaggregates may be a feasible way to increase its stability, while at the same time can help to enhance its beneficial effects by controlling the release to food matrices (Jang and Lee, 2008).

The objective of this work was to evaluate the antioxidant stability and browning of mushroom slices preserved with an edible coating containing AA-loaded chitosan/tripolyphosphate nanoaggregates.

## 2. Materials and methods.

### 2.1. Preparation of ascorbic acid loaded chitosan /tripolyphosphate nanoaggregates.

Chitosan nanoaggregates were prepared according to the procedure reported by Jang and Lee (2008), based on the ionic gelation of chitosan with TPP anions. Two solutions were independently prepared: i) a 0.1 % (w/v) chitosan solution obtained by dissolving intermediate molecular-weight chitosan in a 0.7 % (w/v) ascorbic acid solution, and ii) a 0.1 % (w/v) TPP aqueous solution. The TPP solution was eventually added to the chitosan/ascorbic acid solution under constant stirring (10,000 x g) to reach a ratio of 2:5.

### 2.2. Nanoaggregates characterization.

#### *2.2.1. Particle size and ζ-potential.*

The characterization of the formed nanoaggregates was conducted following the method described by Salvia-Trujillo et al. (2015). The size and ζ-potential of the nanostructured coating-forming solutions were measured by dynamic light scattering (DLS) with a Zetasizer Nano ZS laser diffractometer (Malvern Instruments Ltd., Worcestershire, UK) working at 633 nm at 25 °C and equipped with a backscatter detector (173 °). Samples were diluted (1:10) prior to analysis with acetate buffer (pH=4.5) prepared with Milli-Q water to avoid multiple scattering effects. The particle size distribution of the nanoaggregates was reported as polydispersity index (PDI). The ζ-potential was measured by phase-analysis light scattering (PALS) with a Zetasizer Nano ZS laser diffractometer (Malvern Instruments Ltd., Worcestershire, UK). All measurements were performed in triplicate.

## 2.3. Minimal processing and coating of fresh-cut mushrooms.

Mushrooms (*Agaricus bisporus*) were purchased in a local market. The mushrooms were washed with tap water, dried with paper cloth, cut in slices of 3-5 mm and immediately dipped for one minute in one of the following treatments: a) 0.7 % (w/v) ascorbic acid solution (AAS) b) 0.1 % (w/v) chitosan solution in pH=4.5 acetate buffer (CHS), c) 0.1 % (w/v) chitosan dissolved in a 0.7 % (w/v) ascorbic acid solution (CHS+AA) d) 0.1 % (w/v) chitosan/tripolyphosphate nanoaggregates solution loaded with 0.7 % (w/v) ascorbic acid (NANO) e) water (CONTROL). The ratio product:solution was set at 1:3. The excess of solution was gently drained using a manually operated commercial salad spinner.

Samples of 25 g of mushroom slices were placed in polypropylene trays and thermosealed using an ILPRA Food Pack Basic V/6 packaging machine (ILPRA Systems, CP, Vigevono, Italy). The $O_2$ and $CO_2$ permeability of the sealing film were $5.24 \times 10^{-13}$ mol $m^{-2}$ $s^{-1}$ $Pa^{-1}$ and $2.38 \times 10^{-12}$ mol $m^{-2}$ $s^{-1}$ $Pa^{-1}$ at 23 °C and 0 % RH, respectively (ILPRA Systems Spain, S.L. Mataró, Spain). The packages were stored at 5 °C in darkness for 15 d and four trays were withdrawn every 3 d. Two of them were used for physical determinations and the other two were freeze-dried and stored at -18 °C for further analyses of enzyme activity, antioxidant capacity, phenolic and ascorbic acid contents. All determinations were performed in triplicate.

## 2.4. Quality evaluation of minimally processed mushrooms throughout storage.

### *2.4.1. Package headspace composition*

The composition of the package headspace was determined with a Micro-GC CP 2002 gas analyzer (Chrompack International, Middelburg, The Netherlands) equipped with a thermal conductivity detector, following the methodology proposed by Oms-Oliu et al. (2010). A

sample of 1.7 mL was automatically withdrawn through an adhesive rubber septum with a sampling needle connected to the injection system. The determination of $O_2$ concentrations was carried out by injecting a sample of 0.25 µL into a CP-Molsieve 5 Å column (4 m × 0.35 mm, df = 10 µm) at 60 °C and 100 kPa whereas a sample of 0.33 µL was injected into a PoraPLOT Q column (10 m × 0.32 mm, df = 10 µm) at 75 °C and 200 kPa for $CO_2$. The results were expressed as kPa of $O_2$ and $CO_2$ in the tray headspace.

### *2.4.2. Firmness*

Texture was evaluated using a TA-XT2 texture analyzer (Stable Micro Systems Ltd., Surrey, England, UK) equipped with a Kramer cell. Toughness was measured as the maximum force required for shearing 5 g of sliced mushrooms. The area of the Kramer cell was 9 $cm^2$ and the test speed was 15 mm $s^{-1}$, being the peak stress at 50 % compression. At least 3 measurements per package were carried out. The maximum force achieved at peak stress was expressed as N (Oms Oliu et al., 2010).

### *2.4.3. Color*

Color was measured with a colorimeter (Minolta Chroma Meter Model CR-400, Minolta Sensing Inc., Osaka, Japan). The equipment was set up for a D65 illuminant and a 10 ° observer angle. Four readings were taken on 4 individual slices of each package. CIE L* (lightness), a* (red-green) and b* (yellow-blue) parameters were measured through reflectance values. These values were used to calculate the browning index (BI) (Eq. 1). Changes in BI have previously been proven as effective in monitoring browning of fresh-cut mushrooms (Cliffe-Byrnes and O'Beirne, 2008).

$$BI = 100\,(x - 0.31)/0.17 \qquad \text{Equation (1)}$$

where x = (a* + 1.75·L*)/((5.645·L*) + (a* −(3.012·b*)).

### *2.4.4. Polyphenol oxidase activity*

A sample of 0.2 g of freeze-dried mushrooms was milled to a fine powder and mixed with 15 mL of 50 mM phosphate buffer (pH = 7.0) 1 mM EDTA and 0.25 % (w/v) polyvinylpyrrolidone under magnetic stirring in an ice bath for 30 min. The suspension was centrifuged for 10 minutes at 8,000 x g at 4 °C and the supernatant was filtered. The resulting product constituted the enzyme extract.

To determine the PPO activity, 0.1 mL of properly diluted enzyme extract was mixed with 0.1 mL of 50 mM catechol in 50 mM phosphate buffer (pH = 7.0). The reaction mixture was incubated at 25° C and the absorbance was read every 10 s for 120 s at 420 nm. A unit of PPO activity was equivalent to a variation of 0.001 at 420 nm per minute and it was expressed as U $kg^{-1}$ based on protein content in the enzymatic extracts (Yinsanga et al., 2008).

### *2.4.5. Total phenolic compounds*

Total phenolic compounds were determined on a methanolic extract obtained from freeze-dried mushrooms following the methodology proposed by Ding et al. (2016) with some modifications. Namely, 0.2 g of dried sample was magnetically stirred with 10 mL of 80 % methanol for 2 h in ice bath. The mixture was then filtered through filter paper, and the extract was referred as methanolic extract. The total phenolic content was determined by mixing 12.5 µL of properly diluted extract with 200 µL of distilled water and 12.5 µL of Folin-Ciocalteu reagent. Three minutes later, 25 µL of 7.5 % $Na_2CO_3$ was added. The reaction mixture was left in dark and the absorbance at 760 nm was determined after 60

184 min in a microplate reader (BioTek Instruments Inc., Bad Friedrichshall, Germany). Three
185 replicate analyses were performed, and results were expressed on a dry weight basis as g
186 $kg^{-1}$ of gallic acid equivalents (GAE).
187
188 ***2.4.6. Ascorbic acid content***
189 Ascorbic acid concentrations were determined by HPLC-UV. The extraction procedure and
190 the chromatographic conditions were based on a previous study carried out by Oms-Oliu et
191 al. (2010). A portion of 0.2 g of mushroom powder was added to 5 mL of a solution
192 containing 45 g $L^{-1}$ of metaphosphoric acid and 7.2 g $L^{-1}$ of di- 1, 4-dithiothreitol (DTT).
193 The mixture was stirred and centrifuged at 15,000 ×g for 15 min at 4°C. The supernatant
194 was vacuum-filtered through number 1 Whatman paper. Finally, the sample was passed
195 through a Millipore 0.45 µm membrane before injection into the HPLC system.
196 The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector
197 (Waters, Milford, USA) working at 254 nm. Duplicates of 25 µL of each extract were
198 injected into a reverse phase C18 Spherisorb® ODS2 (5 µm) stainless steel column
199 (250mm×4.6mm) (Waters, Milford, MA), used as stationary phase. A 0.01 % (v/v) sulfuric
200 acid solution adjusted to pH 2.6 was used as mobile phase. The flow rate was fixed at 1 mL
201 $min^{-1}$ at room temperature. Ascorbic acid content was expressed as g $kg^{-1}$.
202
203 ***2.4.7. Antioxidant capacity (AOC)***
204 The antioxidant capacity of minimally processed mushrooms was analyzed using two
205 independent methods (Oms Oliu et al., 2010). The free radical-scavenging effects of the
206 antioxidant extracts on 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2'-azinobis(3-
207 ethylbenzothiazoline)-6-sulphonate (ABTS•+) radicals were determined.

Properly selected dilutions of the previously prepared methanolic extract were mixed with prefixed volumes of freshly prepared of DPPH• and ABTS•+ solutions and allowed to react for one hour. Absorbance was determined at 517 nm and at 734 nm for the DPPH and ABTS assays, respectively, using a microplate reader (BioTek Instruments Inc., Bad Friedrichshall, Germany).

The decrease in absorption was expressed as Trolox equivalent antioxidant capacity (TEAC). TEAC values for both DPPH and ABTS radicals were obtained from standard curves built by measuring the reduction in absorbance at different concentrations of Trolox.

### *2.5. Statistical analysis*

The results were statistically analyzed using analysis of variance (ANOVA) ($\alpha = 0.05$). The differences among means were tested for statistical significance using a multiple-range least significant difference (LSD) test with the Info Stat-Statistical Software 2015 (Córdoba, Argentina).

## 3. Results and discussion

### *3.1. Nanoparticle characterization*

Particle size distribution has been reported to be one of the most significant parameters determining the availability and stability of the encapsulated products. In the present study, ascorbic acid-loaded chitosan/tripolyphosphate nanoaggregates with uniform particle size were successfully produced. The average particle size was 105.72 ± 3.27 nm (Fig. 1), a value similar to that reported by Pilon et al. (2014) for citric acid-loaded chitosan nanoaggregates obtained under similar process conditions. Moreover, the synthesized chitosan/tripolyphosphate nanoaggregates exhibited a monomodal narrow size distribution

pattern with a PDI of 0.267 ± 0.040. Koo et al., (2011) found that the particle size was dependent on the chitosan:TPP ratio, being the particle size significantly increased with higher TPP concentration. The production of nanoaggregates with controlled particle size is usually highly desirable to assure stability and uniform properties (Santiago and Castro, 2016). Small PDI values are associated with narrower size distributions (Lemarchand et al., 2003), PDI values close to 0 exhibit a homogeneous dispersion, characteristic of highly monodisperse systems, whereas higher values indicate that a sample has a broader size distribution. Indeed, values less than 0.7 indicate greater stability for nanodelivery systems. The experimental values suggest that the obtained systems were highly homogeneous regarding its particle size.

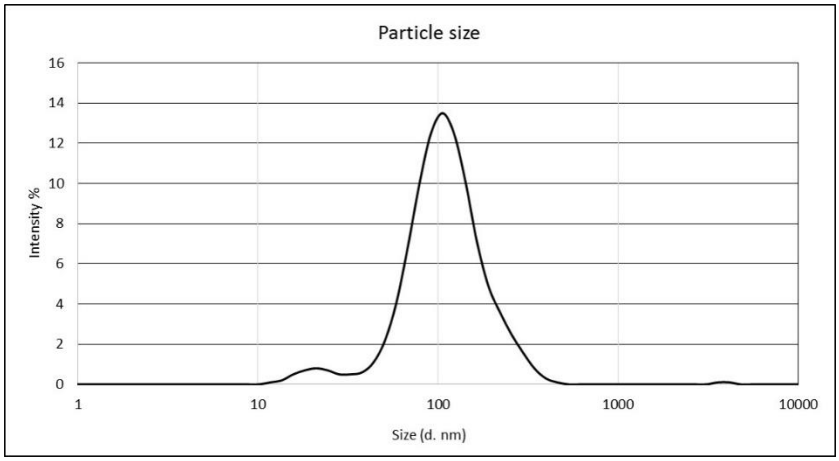


**Figure 1.** Particle size distribution of an edible coating containing AA-loaded chitosan/tripolyphosphate nanoaggregates

The obtained nanoaggregates had an overall positive surface charge with an average zeta potential value of 39.733 ± 0.750 mV. Similar results were reported by Jang and Lee (2008), who found that chitosan and TPP spontaneously form compact nanocomplexes with

250 an overall positive charge when mixed under acidic conditions. Since chitosan is insoluble
251 in water, many of the literature works describe the production of chitosan nanoparticles
252 using acetic acid as solvent (Moura et al., 2009; Jang and Lee, 2008). In the present work,
253 nanoaggregates were prepared with an aqueous ascorbic acid solution, which may be
254 interesting for the development of food-grade applications, as both chitosan and AA are
255 considered to be GRAS substances.
256

257 **3.2. Effect of the application of AA-loaded chitosan/TPP nanoaggregates on quality**
258 **characteristics of mushroom slices.**

259 ***3.2.1. Headspace gas composition.***

260 In-package $O_2$ concentrations progressively decreased during the storage of fresh-cut
261 mushrooms. The reduction of in-package $O_2$ concentration was especially steep over the
262 first days of storage, and subsequently slowed down over time (Table 1). This is in
263 accordance to the fact that major oxidative metabolic reactions occur especially after
264 cutting and that lower $O_2$ concentrations over storage lead to a significant reduction of the
265 respiratory rates of fresh-cut produce (Oms-Oliu et al., 2010). $O_2$ uptake and $CO_2$
266 production appeared to be significantly ($p<0.05$) reduced when AA was used as
267 antibrowning agent (NANO, CHS+AA and AA treatments) (Table 1). In contrast, changes
268 in the in-package concentrations for CONTROL and CHS treatments were more
269 pronounced, leading to lower and higher $O_2$ and $CO_2$ concentrations, respectively. The pH
270 decrease on the surface of mushroom slices caused by AA addition most likely exerted an
271 inhibitory effect on the respiration and oxidative metabolism of the cut product, as
272 previously reported by other authors (Guerrero-Beltrán et al., 2005). Moreover, the use of
273 chitosan as a coating material did not appear to have an influence on the $O_2$ uptake and $CO_2$

274 production. Interestingly, mushroom slices treated with the NANO coating increased the
275 $CO_2$ concentration over the storage more gradually, reaching significantly ($p<0.05$) lower
276 concentrations than those found for any other treatment condition. This may be a
277 consequence of a greater inhibition of the enzymes involved in the phosphorylation
278 pathway. Moreover, a dramatic increase in $CO_2$ production was observed in non-
279 nanostructured chitosan treatments (CHS and CHS+AA), with average values of 23.79 kPa
280 by day three of storage. This could be the result of the increased oxidative metabolism and
281 the direct effect of non-nanostructured chitosan, which has been previously reported to
282 enhance $CO_2$ production (Lee et al., 2005). It is known that the abiotic stress caused by
283 chitosan coatings could activate enzymes such as chitinases, phenylalanine ammonia-lyase
284 (PAL) and peroxidases; leading to an increase in $CO_2$ levels (Simoes et al., 2009).
285 Nevertheless, our results suggest that the use of chitosan/tripolyphosphate nanoaggregates
286 on fresh-cut mushrooms could help to avoid these undesirable effects.
287
288 ***3.2.2. Firmness.***
289 AA-loaded nanostructured chitosan-tripolyphosphate coatings (NANO) best preserved the
290 initial firmness values of fresh-cut mushrooms (Table 1) throughout storage ($p>0.05$).
291 Similarly, the firmness of fresh-cut mushrooms coated with CHS+AA did not noticeably
292 deplete over storage.
293 These observations contrast with the significant ($p<0.05$) reduction of initial firmness
294 occurring in CONTROL, CHS- and AAS-treated mushrooms, with values averaging 22.02
295 ± 1.40 N (Table 1). Immersion in water-based diluted solutions has been reported to
296 dramatically affect the textural properties of fresh-cut mushrooms due to water absorption
297 (Sapers et al., 1994; 2001). Firmness of those samples subsequently increased after 3 d and

through the remaining storage period, reaching final values of 29.21 and 31.03 N. Firmness changes are the consequence of the promotion of complex enzymatic (Sahai and Manocha, 1993) and water transfer phenomena (Yurttas et al., 2013). During immersion treatments, the superficial cellular structures of the mushrooms are affected, and structural changes are promoted. As a consequence, the cells become more fragile, water loss is promoted and firmness increases (Hernando et al., 2008).

### *3.2.3. Color and overall appearance*

Ascorbic acid effectively helped to control browning of fresh-cut mushrooms immediately after processing regardless the kind of treatment, as was evidenced by the low initial BI values. The use of 0.7 % ascorbic acid combined with nanostructured chitosan (NANO) reduced browning development during storage and significantly contributed to maintain the overall visual quality. It is important to highlight that this concentration of ascorbic acid is significantly lower than those conventionally used as antibrowning treatments in other studies (Sapers et al., 1994; Yurttas et al., 2013). The BI values of mushrooms coated with the NANO solution did not significantly change over 12 d of storage and only increased by 36 % at day 15 (Table 1), were signs of decay began to appear. Indeed, BI values were significantly ($p < 0.05$) lower than those of fresh-cut mushrooms subjected to any other treatment, thus indicating that the antibrowning effect of ascorbic acid was extended when loaded into nanoaggregates. This contrasts with color changes observed in mushrooms dipped in ascorbic acid (AAS treatment) or coated with chitosan (CHS+AA), whose BI values dramatically increased after the first few days of storage. Interestingly, non-nanostructured chitosan coatings, either alone (CHS) or combined with AA (CHS+AA), did not successfully prevent browning of mushrooms slices. Hence, BI values of mushrooms

coated with non-structured chitosan were significantly higher than those observed in any other case, even in CONTROL samples. In addition, signs of microbiological decay were evident in CHS-coated samples beyond the first week of storage. These results are likely a consequence of the promotion of oxidative metabolism. Chitosan could trigger abiotic stress acting as chemical elicitor, increasing browning reactions and phenylpropanoids metabolism, and hence promoting brown color development (Lee et al., 2005; Simoes et al., 2009)

**Table 1.** Changes in browning index (BI), firmness and gas headspace composition of mushroom slices during refrigerated storage.

| Treatment | Time (days) | $O_2$ (kPa) | $CO_2$ (kPa) | Firmness (N) | BI |
|---|---|---|---|---|---|
| CONTROL | 0.08 | 16.38 ± 0.25 Aa | 3.06 ± 1.72 Aa | 26.43 ± 1.19 Aca | 17.12 ± 2.04 Aa |
| | 3 | 8.86 ± 0.36 Ab | 16.99 ± 0.97 Bb | 23.22 ± 1.12Ab | 16.79 ± 1.97 Aa |
| | 6 | 7.40 ± 0.32 Ac | 20.76 ± 1.83 Bb | 23.01 ± 1.13 Ab | 22.15 ± 1.68 Aa |
| | 9 | 4.74 ± 1.17 Ac | 26.89 ± 1.23 Bc | 23.29 ± 0.88 Ab | 26.01 ± 1.68 Ab |
| | 12 | 4.70 ± 1.13 Ac | 28.00 ± 1.10 Bc | 26.31 ± 0.88 Aa | 27.31 ± 2.67 Ab |
| | 15 | 4.97 ± 1.10 Ac | 31.50 ± 1.10 Bc | 29.21 ± 0.93 Ac | 26.31 ± 2.73 Ab |
| CHS | 0.08 | 14.63 ± 0.51 Aa | 2.43 ± 1.12 Aa | 21.79 ± 1.35 Ba | 43.40 ± 3.77 Ba |
| | 3 | 9.37 ± 0.77 Ab | 24.52 ± 1.72 Cb | 21.04 ± 1.15 Aa | 57.24 ± 4.95 Bb |
| | 6 | 5.20 ± 1.06 Ac | 32.07 ± 2.09 Cc | 21.42 ± 1.07 Ba | 56.32 ± 3.68 Bb |
| | 9 | 4.62 ± 0.27 Ac | 31.07 ± 1.71 BCc | 26.07 ± 1.38 Bb | 54.55 ± 3.12 Bb |
| | 12 | 4.74 ± 0.42 Ac | 32.43 ± 1.22 Cc | 29.05 ± 1.08 Bc | 58.52 ± 2.30 Bb |
| | 15 | 5.14 ± 0.82 Ac | 31.65 ±2.16 Bc | 29.78 ± 0.86 Ac | 75.77 ± 2.44 Bc |

| | | | | | |
|---|---|---|---|---|---|
| AAS | 0.08 | 19.32 ± 0.87 Ba | 3.08 ± 1.96 Aa | 22.25 ± 1.46 Ba | 10.04 ± 2.28 Aa |
| | 3 | 15.16 ± 0.81 Bb | 11.43 ± 0.69 Db | 23.69 ± 1.38 Aa | 11.80 ± 2.96 Aa |
| | 6 | 11.63 ± 0.84 Bc | 13.68 ± 1.22 Db | 26.12 ± 0.74 Cb | 21.67 ± 3.32 Ab |
| | 9 | 9.86 ± 0.39 Bd | 23.45 ± 1.07 Bc | 26.17 ± 0.43 Bb | 28.42 ± 2.86 Ac |
| | 12 | 8.73 ± 0.51 Bd | 28.00 ± 0.61 Bc | 29.07 ± 1.82 Bc | 28.41 ± 2.39 Ac |
| | 15 | 7.14 ± 1.09 Ad | 31.85 ± 0.98 Bd | 31.03 ± 1.31 Aa | 27.62 ± 1.75 Ac |
| CHS+AA | 0.08 | 18.38 ± 0.72 Ba | 3.20 ± 1.37 Aa | 24.52 ± 1.04 Aa | 12.59 ± 2.91 Aa |
| | 3 | 16.04 ± 0.82 Ba | 23.07 ± 0.97 Cb | 24.05 ± 1.28 Aa | 29.35 ± 3.33 Cb |
| | 6 | 11.37 ± 1.01 Bb | 27.98 ± 1.39 Cb | 25.31 ± 1.10 Ca | 29.63 ± 2.50 Cb |
| | 9 | 7.98 ± 0.37 Bc | 28.99 ± 1.35 BCb | 26.38 ± 0.83 Ba | 39.64 ± 1.47 Cc |
| | 12 | 8.19 ± 0.13 Bc | 31.11 ± 0.93 Cbc | 26.95 ± 0.95 Aa | 37.24 ± 3.81 Cc |
| | 15 | 6.76 ± 0.50 Ac | 32.56 ± 0.95 Bc | 26.99 ± 1.32 Ba | 41.06 ± 3.55 Cc |
| NANO | 0.08 | 17.82 ± 1.29 Ba | 2.71 ± 0.83 Aa | 23.51 ± 1.37 ABa | 10.28 ± 2.43 Aa |
| | 3 | 16.13 ± 0.94 Ba | 11.67 ± 1.72 Db | 23.26 ± 1.18 Aa | 9.68 ± 1.96 Da |
| | 6 | 12.76 ± 0.70 Bb | 16.49 ± 0.95 Db | 23.89 ± 0.92 Aa | 9.40 ± 0.81 Da |
| | 9 | 10.96 ± 1.26 Bb | 22.23 ± 1.11 Bc | 24.47 ± 1.53 Ba | 11.73 ± 1.18 Da |
| | 12 | 7.85 ± 0.51 Bbc | 25.67 ± 1.10 Bc | 24.05 ± 1.86 Ca | 12.04 ± 2.59 Da |
| | 15 | 6.88 ± 1.09 Ac | 29.29 ± 1.49 Cd | 24.72 ± 1.64 Ca | 18.82 ± 3.97 Db |

Values followed by different capital letters within a column indicate statistically significant differences ($p<0.05$) between treatments for a same storage time. Values are expressed as mean ± standard deviation.

Values followed by different lowercase letters within a column indicate statistically significant differences ($p<0.05$) between values obtained at different storage times for a same treatment. Values are expressed as mean ± standard deviation.

CHS: chitosan solution, AAS: ascorbic acid solution, CHS+AA: chitosan + ascorbic acid, NANO: ascorbic acid-loaded chitosan/tripolyphosphate nanoaggregates.

### *3.2.4. PPO activity*

Initial PPO activity values ranged between $11.60 \times 10^7$ and $13.48 \times 10^7$ U $kg^{-1}$ in mushrooms treated under any of the assayed treatment conditions ($p < 0.05$), with the exception of those only coated with chitosan (CHS treatment). By day three, a peak in PPO activity was observed in all the samples. At that point, the lowest activity was found in mushrooms slices coated with AA-loaded chitosan/tripolyphosphate coatings (NANO), and the highest one in CONTROL mushroom slices (Figure 2). PPO activity subsequently decreased reaching values close to the initial values for NANO and CHS+AA treatments. The PPO activity increase during the early stages of storage of mushroom slices is most likely the consequence of the tissue response to mechanical injury. The initial increase in enzymatic activity has been largely reported and is known to be related, on the one hand, to the abrupt release of enzymes accumulated in vacuoles, and, on the other hand, to the increased enzyme synthesis triggered by physical damage (Guerrero-Beltrán et al., 2005). The incorporation of ascorbic acid to chitosan formulations (NANO and CHS+AA) led to significantly lower PPO activity values through the whole storage period in comparison to other treatments. AA has been reported to exert a direct inhibitory effect of some reaction products on the enzyme's catalytic site (Lamikanra et al., 2000). The lower PPO activity values observed in mushrooms coated with AA-loaded chitosan/tripolyphosphate coatings (NANO) could be related to the better preservation of initial AA concentration. Nano-encapsulation protects AA from oxidation, therefore its degradation is delayed, and its anti-browning effect is promoted (Desai and Park, 2005; Jang and Lee, 2008). AA can be loaded

into the nanoaggregates through three different mechanisms: (1) electrostatic interaction, (2) encapsulation, and (3) adsorption (Alishahi et al., 2011).

In contrast mushrooms coated only with chitosan (CHS) exhibited a higher initial activity of 22.23 ± 1.37 x $10^7$ U $kg^{-1}$ (Fig. 2). This enhanced enzymatic activity could be linked to the fact that the pH of the chitosan solution is close to the optimum pH of tyrosinase activity (Ratcliffe et al., 1994). Non-nanostructured chitosan has also been reported to promote abiotic stress-related responses, leading to the synthesis of phenolic compounds and PPO (Simoe et al., 2009).

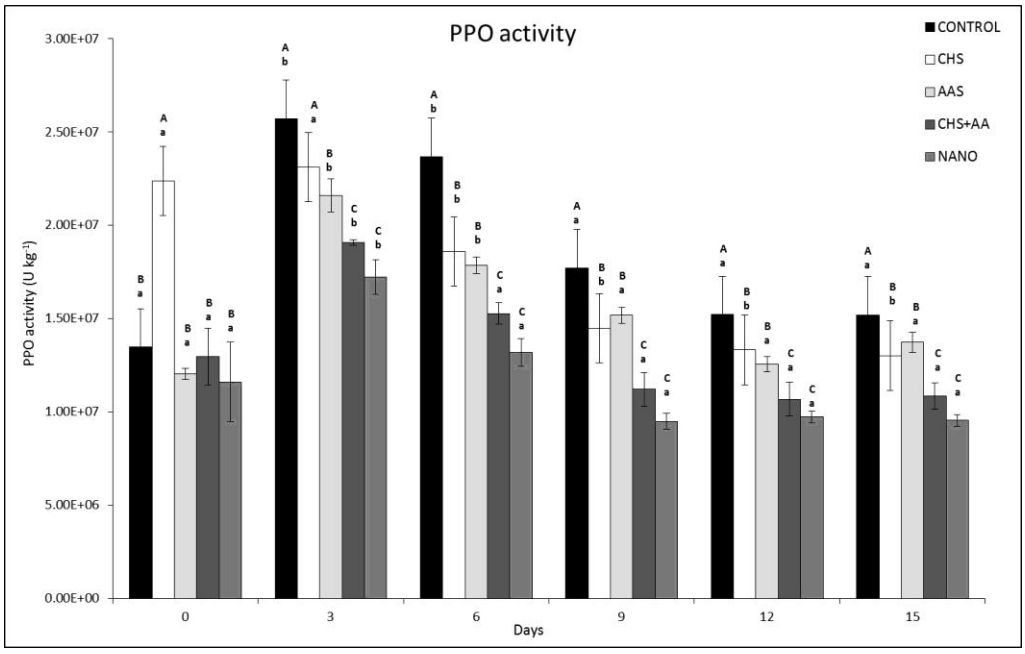


**Figure 2.** PPO activity of mushroom slices during cold storage. Values are expressed as mean ± SD. Capital letters indicate differences ($p<0.05$) between treatments for the same storage time while lowercase letters indicates differences ($p<0.05$) during storage within a treatment. CHS: chitosan solution, AAS: ascorbic acid solution, CHS+AA: chitosan + ascorbic acid, NANO: ascorbic acid loaded chitosan/tripolyphosphate nanoaggregates.

### *3.2.5. Phenolic compounds*

Fresh-cut mushrooms treated with ascorbic acid (NANO, CHS+AA and AAS) exhibited a much higher phenolic content than CONTROL and CHS treated mushrooms (Fig. 3).

During refrigerated storage, the phenolic content of mushroom slices coated with AA-loaded chitosan/tripolyphosphate nanoaggregates (NANO) slightly decreased. By day six of storage 18 % of the initial phenolic content was lost but remained without further significant reduction. No significant differences between coatings containing chitosan and ascorbic acid (NANO and CHS+AA) were observed. The effect of ascorbic acid in phenolic content is probably the result of the combined effects of its reducing nature, and its well-reported interference with the spectrometric determination, and its inhibitory effect on PPO and PAL activities. On the other hand, phenolic content significantly increased in CONTROL samples, which could be associated to the triggering of secondary metabolic pathways as a response to mechanical injury.

Interestingly, mushrooms coated with chitosan without added AA (CHS) exhibited a significantly lower phenolic content than CONTROL mushrooms throughout the whole storage period. Phenolic content in the CHS-coated mushrooms slightly increased just after processing and peaked at day 3, probably as a consequence of the wounding response. The content slightly decreased subsequently and remained stable, which suggests that the initial synthesis of phenolic compounds was compensated by enzymatic oxidation, as evidenced by major browning phenomena occurring on those samples.

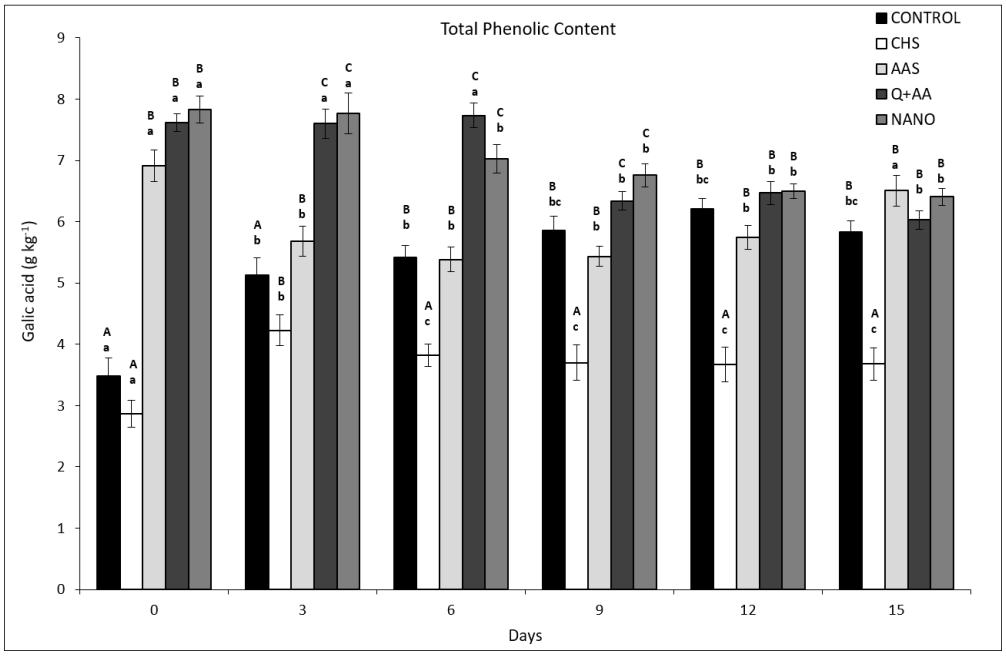


**Figure 3.** Changes in total phenolic content in sliced mushrooms during cold storage. Values are expressed as mean ± SD. Capital letters indicate differences ($p<0.05$) between treatments for the same storage time while lowercase letters indicates differences ($p<0.05$) during storage within a treatment. CHS: chitosan solution, AAS: ascorbic acid solution, CHS+AA: chitosan + ascorbic acid, NANO: ascorbic acid loaded chitosan/tripolyphosphate nanoaggregates.

### *3.2.6. Ascorbic acid content*

The ascorbic acid content of fresh mushrooms was 1.84 ± 0.18 g $kg^{-1}$. This value falls within the values reported by other authors (Barros et al., 2008; Matila et al., 2001). When ascorbic acid treatments were applied, initial contents were up to 60 % higher than those found in CONTROL mushroom slices, being 2.98 ± 0.24, 2.97 ± 0.17 and 2.92 ± 0.11 g $kg^{-1}$ for NANO, CHS+AA and AAS respectively (Table 2).

Mushrooms preserved with AA-loaded nanostructured chitosan/tripolyphosphate coatings (NANO) best preserved their initial AA content. No significant reductions were observed during the first 6 d of storage and at least a 65 % of the initial content was retained further

416 on. Similarly, CHS+AA slices exhibited a similar trend but with a more significant
417 degradation of AA through storage (Table 2), which evidences the protective effect of
418 nano-encapsulation.
419 In contrast, AA concentrations dramatically decreased in mushrooms dipped in an AA
420 solution (AAS treatment), hence reaching a 19 % of the initial content at 15 d of storage.
421 This significant loss is likely the consequence of oxidation by reactive oxygen species and
422 the reduction of products of enzymatic oxidative reactions (Gorny et al., 2002) (Table 2).
423 Similarly, less than 9 % of the initial AA content remained in untreated mushroom slices
424 (CONTROL) after 15 d of storage, while CHS-coated mushrooms slices maintained a 33 %
425 of their initial content. These results clearly illustrate the protective effect of non-
426 nanostructured chitosan on the AA content of fresh-cut mushrooms. Likewise, a similar
427 effect has been previously reported for other polysaccharide-based coatings in other food
428 matrices (Ojeda et al., 2014).
429 The results show that the use of chitosan/tripolyphosphate nanoaggregates improves the
430 stability of AA. As previously reported by Jang and Lee (2008), who evaluated the thermal
431 stability of ascorbic acid when loaded into nanoaggregates, the chitosan-tripolyphosphate
432 structure is able to surround AA, thus preventing its direct contact with environmental
433 factors such as light, heat and oxygen and extending its shelf-life.
434
435 ***3.2.7. Antioxidant capacity (AOC)***
436 ABTS and DPPH radical scavenging capacities of fresh-cut mushrooms were consistent for
437 all assayed treatment, thus exhibiting similar trends throughout storage (Table 2). DPPH
438 values were significantly higher than ABTS values, although the antioxidant capacity
439 values by both assays were strongly correlated. The specificity of each radical to

antioxidant compounds in the assay condition could explain these differences. DPPH radical is generally considered to be less specific than ABTS. For instance, ABTS has shown to be better more specific against phenolic compounds, as it is applicable to both hydrophilic and lipophilic antioxidant systems (Floegel et al., 2011). Particularly, it was reported that DPPH is more sensitive to the antioxidant effect of ascorbic acid when compared to ABTS (Kim et al., 2002).

The initial total ABTS and DPPH radical-scavenging activities for NANO coated mushrooms were $9.99 \pm 0.32 \times 10^4$ µmol $kg^{-1}$ and $6.27 \pm 0.27 \times 10^5$µmol $kg^{-1}$ respectively. This AOC values were significantly higher than those determined in any other sample. The AOC in NANO coated mushrooms slightly decreased during storage, however, after 6 d no significant changes were detected with the ABTS assay. With NANO treatments, the protective effect of nano-encapsulation avoided AA losses, therefore the AOC decreases were reduced or minimized (< 25 % for DPPH assay). A descending trend was observed in CHS+AA and AAS mushrooms, being higher than 28 % at day fifteen for both assays. The differences detected between the applied treatments are consistent with the protective effect of chitosan nanoaggregates on ascorbic acid found in this study and previously reported by other authors (Desai and Park, 2005).

On the other hand, mushroom slices coated with chitosan without added AA (CHS) exhibited the lowest initial AOC values (Table 2). These values slightly increased by 27 and 47 % ABTS and DPPH, respectively, probably as a consequence of the reducing effect of some quinones formed during early stages of browning development (Sapers et al., 1994; 2001). As well, AOC values significantly ($p<0.05$) increased in uncoated mushrooms slices over storage, reaching values that were 61 and 32 % higher than those found just after processing using the ABTS and DPPH methods, respectively. This increase in the AOC of

464 fresh cut mushrooms has been previously reported by other authors (Oms Oliu et al., 2011,
465 Ding et al., 2016), and is in line with the increase in the phenolic content.
466 Although some authors have reported a direct correlation between mushroom AOC and
467 total phenolic content, in the present work, the AOC is a consequence of the addition of
468 ascorbic acid. On the other hand, the increases of AOC in CONTROL and CHS
469 mushrooms are probably due to the higher values of total phenols, although the AOC also
470 can be raised by other substances such as tocopherols and carotene (Barros et al., 2008;
471 Kim et al., 2006).
472
473 **Table 2.** Ascorbic acid content and antioxidant capacity (AOC) evaluated by DPPH and ABTS
474 assays in mushrooms slices during refrigerated storage. Results are expressed in dry weight base.

| Treatment | Time | Ascorbic acid (g $kg^{-1}$) | ABTS x $10^4$ (µmol $kg^{-1}$) | DPPH x $10^5$ (µmol $kg^{-1}$) |
|---|---|---|---|---|
| CONTROL | 0 | 1.84 ± 0.18 Ba | 3.54 ± 0.37 Aa | 2.69 ± 0.28 Ba |
| | 3 | 0.85 ± 0.11 Cb | 5.20 ± 0.28 Ab | 2.66 ± 0.17 Ba |
| | 6 | 0.79 ± 0.16 Bb | 5.36 ± 0.38 Ab | 2.63 ± 0.28 Ba |
| | 9 | 0.65 ± 0.06 CDbc | 5.05 ± 0.12 Ab | 3.55 ± 0.11 Ba |
| | 12 | 0.20 ± 0.04 Dd | 5.06 ± 0.28 Ab | 3.54 ± 0.28 Ab |
| | 15 | 0.17 ± 0.06 Dd | 5.72 ± 0.22 Ab | 3.56 ± 0.24 Ab |
| CHS | 0 | 1.02 ± 0.25 Ca | 3.08 ± 0.39 Aa | 1.62 ± 0.24 Aa |
| | 3 | 0.62 ± 0.16 Cb | 3.99 ± 0.22 Bb | 1.62 ± 0.14 Aa |
| | 6 | 0.63 ± 0.13 Cb | 3.98 ± 0.24 Bb | 1.70 ± 0.29 Aa |
| | 9 | 0.62 ± 0.15 Cb | 3.83 ± 0.35 Bb | 2.37 ± 0.39 Ab |
| | 12 | 0.39 ± 0.08 CDc | 4.17 ± 0.23 Ab | 2.38 ± 0.26 Ab |

| | | | | |
|---|---|---|---|---|
| | 15 | 0.34 ± 0.05 CDc | 3.92 ± 0.29 Bb | 2.39 ± 0.24 Ab |
| AAS | 0 | 2.92 ± 0.11 Aa | 7.91 ± 0.48 Ba | 4.52 ± 0.23 Ca |
| | 3 | 1.67 ± 0.10 Bb | 7.62 ± 0.43 Ca | 3.40 ± 0.13 Bb |
| | 6 | 0.96 ± 0.11 Bc | 5.93 ± 0.37 Ab | 3.86 ± 0.17 Bb |
| | 9 | 0.94 ± 0.18 BCc | 5.78 ± 0.31 Ab | 3.50 ± 0.77 Bb |
| | 12 | 0.65 ± 0.09 Cd | 5.88 ± 0.29 Ab | 3.00 ± 0.11 Ac |
| | 15 | 0.58 ± 0.21 Cd | 5.68 ± 0.28 Ab | 2.51 ± 0.24 Ac |
| CHS+AA | 0 | 2.97 ± 0.17 Aa | 9.66 ± 0.40 Ca | 5.86 ± 0.19 Da |
| | 3 | 2.77 ± 0.19 Aa | 9.55 ± 0.18 Da | 5.27 ± 0.18 Ca |
| | 6 | 1.81 ± 0.10 Ab | 8.35 ± 0.21 Ca | 5.15 ± 0.21 Ca |
| | 9 | 1.12 ± 0.16 Bc | 6.61 ± 0.23 Cb | 3.41 ± 0.14 Bb |
| | 12 | 1.14 ± 0.10 Bc | 6.54 ± 0.29 Bb | 3.45 ± 0.15 Ab |
| | 15 | 1.05 ± 0.12 Bc | 5.36 ± 0.32 Ab | 3.23 ± 0.16 Ab |
| NANO | 0 | 2.98 ± 0.24 Aa | 9.99 ± 0.32 Ca | 6.27 ± 0.27 Da |
| | 3 | 2.72 ± 0.17 Aa | 9.55 ± 0.22 Da | 6.36 ± 0.25 Da |
| | 6 | 1.96 ± 0.18 Ab | 8.89 ± 0.25 Cb | 6.43 ± 0.17 Da |
| | 9 | 1.95 ± 0.16 Ab | 8.24 ± 0.24 Db | 5.66 ± 0.99 Cb |
| | 12 | 1.53 ± 0.16 Ab | 8.26 ± 0.31 Cb | 5.68 ± 0.15 Bb |
| | 15 | 1.49 ± 0.12 Ab | 8.09 ± 0.25 Cb | 4.64 ± 0.21 Bc |

475 Values followed by different capital letters within a column indicate statistically significant
476 differences ($p<0.05$) statically differences between treatments for a same storage time. Values are
477 expressed as mean ± standard deviation.
478 Values followed by different lowercase letters within a column indicate statistically significant
479 differences ($p<0.05$) between values measured at different storage times for a same treatment.
480 Values are expressed as mean ± standard deviation.

CHS: chitosan solution, AAS: ascorbic acid solution, CHS+AA: chitosan + ascorbic acid, NANO: ascorbic acid loaded chitosan/tripolyphosphate nanoaggregates.

## 4. Conclusions

Coatings containing AA-loaded chitosan/tripolyphosphate nanoaggregates successfully contributed to extend the postharvest quality of minimally processed mushrooms at 5 °C. The results indicate that the nanostructured coating can effectively alleviate browning development and maintain firmness. More than a 50 % of the initial ascorbic acid levels was preserved for at least 15 d of refrigerated storage. Furthermore, higher phenolic content and antioxidant capacity values were achieved thanks to nano-encapsulated ascorbic acid dips. Polyphenol oxidase activity was partially inhibited as a consequence of the high AA concentration maintained during storage. The product shelf-life considering visual quality could be estimated in at least 12 d. Thus, AA-loaded chitosan/tripolyphosphate nanoaggregates are a feasible alternative to prevent browning of fresh-cut mushrooms and maintain high ascorbic acid concentrations through refrigerated storage. Further research should be conducted to evaluate the impact of the product on human health and the environment, in accordance to the regulations imposed by different regulatory authorities.

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## 6. References


Alishahi, A., Mirvaghefi, A., Tehrani, M.R., Farahmand, H., Shojaosadati, S.A., Dorkoosh, F.A., Elsabee, M.Z. 2011. Shelf life and delivery enhancement of vitamin C using chitosan nanoparticles. Food Chem. 126, 935–940.

Barros, L., Cruz, T., Baptista, P., Estevinho, L.M., Ferreira, I.C.F.R. 2008. Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food Chem. Toxicol. 46, 2742–2747.

Beaulieu, M., D'Aprano, G., Lacroix, M. 2002. Effect of dose rate of gamma irradiation on biochemical quality and browning of mushrooms *Agaricus bisporus*. Radiat. Phys. Chem. 63, 311–315.

Cliffe-Byrnes, V., O'Beirne, D.O. 2008. Effects of washing treatment on microbial and sensory quality of modified atmosphere (MA) packaged fresh sliced mushroom (*Agaricus bisporus*).Postharvest Biol. Tech. 48, 283–294.

Desai, K.G.H., Park, H.J. 2005. Encapsulation of vitamin C in tripolyphosphate cross-linked chitosan microspheres by spray drying. J. microencapsul. 22, 179–92.

Ding, Y., Zhu, Z., Zhao, J., Nie, Y., Zhang, Y., Sheng, J., Meng, D., Mao, H., Tang, X. 2016. Effects of Postharvest Brassinolide Treatment on the Metabolism of White Button Mushroom (*Agaricus bisporus*) in Relation to Development of Browning During Storage. Food Bioprocess. Technol. 9, 1327–1334.

Floegel, A., Kim, D.O., Chung, S.J., Koo, S.I., Chun, O.K. 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J. Food Compos. Anal. 24, 1043–1048.

527 Gorny, J.R., Hess-Pierce, B., Cifuentes, R.A., Kader, A.A. 2002. Quality changes in fresh-
528 cut pear slices as affected by controlled atmospheres and chemical preservatives.
529 Postharvest Biol. Tech. 24, 271–278.
530 Guerrero-Beltrán, J., Swanson, B., Barbosa-Cánovas, G.V. 2005. Inhibition of
531 polyphenoloxidase in mango puree with 4-hexylresorcinol, cysteine and ascorbic acid.
532 LWT- Food Sci. Technol. 38, 625–630.
533 Hernando, I., Sanjuán, N., Pérez-Munuera, I., Mulet, A. 2008. Rehydration of freeze-dried
534 and convective dried Boletus edulis mushrooms: Effect on some quality parameters. J.
535 Food Sci. 73, 356-362.
536 Jang, K.I., Lee, H.G. 2008. Stability of Chitosan Nanoparticles for L -Ascorbic Acid during
537 Heat Treatment in Aqueous Solution. J. Agr. Food Chem. 56, 1936–1941.
538 Jolivet, S., Arpin, N., Wichers, H.J., Pellon, G. 1998. *Agaricus bisporus* browning: a
539 review. Mycol. Res. 102, 1459–1483.
540 Kim, D.O., Lee, K.W., Lee, H.J., Lee, C.Y. 2002. Vitamin C equivalent antioxidant
541 capacity (VCEAC) of phenolic phytochemicals. J. Agr. Food Chem. 50, 3713–3717.
542 Kim, K.M., Ko, J.A., Lee, J.S., Park, H.J., Hanna, M.A. 2006. Effect of modified
543 atmosphere packaging on the shelf-life of coated, whole and sliced mushrooms. LWT-
544 Food Sci. Technol. 39, 364–371.
545 Koo, S.H., Lee, J.S., Kim, G.H., Lee, H.G. 2011. Preparation, characteristics, and stability
546 of glutathione-loaded nanoparticles. J. Agri. Food Chem. 59, 11264–11269.
547 Lamikanra, O., Chen, J.C., Banks, D., Hunter, P.A. 2000. Biochemical and microbial
548 changes during the storage of minimally processed cantaloupe. . J. Agri. Food Chem. 48,
549 5955–5961.

550 Lee, Y.S., Kim, Y.H., Kim, S.B. 2005. Changes in the respiration, growth, and vitamin C
551 content of soybean sprouts in response to chitosan of different molecular weights. Horti.c
552 Sci. 40, 1333–1335.
553 Lemarchand, C., Couvreur, P., Vauthier, C., Costantini, D., Gref, R. 2003. Study of
554 emulsion stabilization by graft copolymers using the optical analyzer Turbiscan. Int. J.
555 Pharm. 254, 77–82.
556 Mohapatra, D., Bira, Z.M., Kerry, J.P., Frías, J.M., Rodrigues, F.A. 2010. Postharvest
557 hardness and color evolution of white button mushrooms (*Agaricus bisporus*). J. Food Sci.
558 75, 146–152.
559 Moura, M.R. de, Aouada, F.A., Avena-Bustillos, R.J., McHugh, T.H., Krochta, J.M.,
560 Mattoso, L.H.C. 2009. Improved barrier and mechanical properties of novel hydroxypropyl
561 methylcellulose edible films with chitosan/tripolyphosphate nanoparticles. J. Food Eng. 92,
562 448–453.
563 Murcia, M.A., López-Ayerra, B., Martinez-Tomé, M., Vera, A.M., García-Carmona, F.
564 2000. Evolution of ascorbic acid and peroxidase during industrial processing of broccoli. J.
565 Sci. Food Agr. 80, 1882–1886.
566 Oms Oliu, G., Aguiló-Aguayo, I., Martín-Belloso, O., Soliva-Fortuny, R. 2010. Effects of
567 pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms
568 (*Agaricus bisporus*). Postharvest Biol. Tech. 56, 216–222.
569 Piagentini, A.; Martin, L.; Bernardi, C.; Güemes, D.; Pirovani, M. 2012. Colour changes in
570 fresh-cut fruits as affected by cultivar, chemical treatment, storage time and temperature.
571 In: Colour in Food: Technological and Psychophysical Aspects. Editorial CRC Press, Boca
572 Raton, USA. Edited by J.L. Caivano and M.P. Buera. Pp. 263–270.

573 Pilon, L., Spricigo, P.C., Miranda, M., Moura, M.R. de, Assis, O.B.G., Mattoso, L.H.C.,
574 Ferreira, M.D. 2014. Chitosan nanoparticle coatings reduce microbial growth on fresh-cut
575 apples while not affecting quality attributes. Int. J. Food Sci. Tech. 50, 440–448.
576 Ratcliffe, B., Flurkey, W.H., Kuglin, J., Dawley, R. 1994. Tyrosinase, Laccase, and
577 Peroxidase in Mushrooms. J. Food Sci. 59, 824–827.
578 Sahai, A. S., Manocha, M.S. 1993. Chitinases of fungi and plants: Their involvement in
579 morphogenesis and host-parasite interaction. FEMS Microbiol. Rev. 11, 317–338.
580 Salvia-Trujillo, L., Rojas-Graü, M.A., Soliva-Fortuny, R., Martín-Belloso, O. 2015. Use of
581 antimicrobial nanoemulsions as edible coatings: Impact on safety and quality attributes of
582 fresh-cut fuji apples. Postharvest Biol. Tech. 105, 8–16.
583 Santiago, L.G., Castro, G.R. 2016. Novel technologies for the encapsulation of bioactive
584 food compounds. Curr.Opi. Food Sci. 7, 78–85.
585 Sapers, G.M., Miller, R. L., Pilizota, V., Kamp, F. 2001. Shelf-Life Extension of Fresh
586 Mushrooms (*Agaricus bisporus*) By Application of Hydrogen Peroxide and Browning
587 Inhibitors. J. Food Sci. 66, 362–366.
588 Sapers, G.M., Miller, R.L., Miller, F.C., Cooke, P.H., Choi, S. 1994. Enzymatic Browning
589 Control in Minimally Processed Mushrooms. J. Food Sci.59, 1042–1047.
590 Simoes, A.D.N., Tuedela, J.A., Allende, A., Puschmann, R., Gil, M.I. 2009. Edible coatings
591 containing chitosan and moderate modified atmospheres maintain quality and enhance
592 phytochemicals of carrot sticks. Postharvest Biol. Tech. 51, 364–370.
593 Singh, P., Langowski, H.C., Wani, A.A., Saengerlaub, S. 2010. Recent advances in
594 extending the shelf life of fresh Agaricus mushrooms: A review. J. Sci. Food Agr. 90,
595 1393–1402.

596 Soliva-Fortuny, R. C., Elez-Martinez, P., Sebastian-Caldero, M., Martin-Belloso, O. 2002.
597 Kinetics of polyphenol oxidase activity inhibition and browning of avocado purée
598 preserved by combined methods. J. Food Eng. 55, 131-137.
599 Taghizadeh, M., Gowen, A., Ward, P., O'Donnell, C.P. 2010. Use of hyperspectral imaging
600 for evaluation of the shelf-life of fresh white button mushrooms (*Agaricus bisporus*) stored
601 in different packaging films. Innov. Food Sci. Emerg. 11, 423–431.
602 Yinsanga, P., Srilaong, V., Kanlayanarat, S., Noichinda, S., McGlasson, W.B. 2008.
603 Relationship between browning and related enzymes (PAL, PPO and POD) in rambutan
604 fruit (*Nephelium* Linn.) cvs. Rongrien and See-Chompoo. Postharvest Biol. Tech. 50, 164–
605 168.
606 Yuk, H.G., Yoo, M.Y., Yoon, J.W., Marshall, D.L., Oh, D.H. 2007. Effect of combined
607 ozone and organic acid treatment for control of Escherichia coli O157:H7 and *Listeria*
608 *monocytogenes* on enoki mushroom. Food Control, 18, 548–553.
609 Yurttas, Z.S., Moreira, R.G., Castell-Perez, E. 2013. Combined vacuum impregnation and
610 electron-beam irradiation treatment to extend the storage life of sliced white button
611 mushrooms (*Agaricus bisporus*). J. Food Sci. 79, 39-46.